

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
8 July 2004 (08.07.2004)

PCT

(10) International Publication Number
WO 2004/057018 A3

(51) International Patent Classification⁷: **C07K 14/21**,
14/26, C12Q 1/18, 1/68, A61K 39/104, 39/108

Christian [CH/CH]; 24, chemin David-Munier, CH-1223
Cologny (CH).

(21) International Application Number:
PCT/CH2003/000836

(74) Agent: E. BLUM & CO.; Vorderberg 11, CH-8044 Zürich
(CH).

(22) International Filing Date:
19 December 2003 (19.12.2003)

(81) Designated States (*national*): AE, AG, AL, AM, AT, AU,
AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR,
CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD,
GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR,
KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN,
MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU,
SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA,
UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
10/324,967 20 December 2002 (20.12.2002) US

(84) Designated States (*regional*): ARIPO patent (BW, GH,
GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW),
Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM),
European patent (AT, BE, BG, CH, CY, CZ, DE, DK, EE,
ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE,
SI, SK, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA,
GN, GQ, GW, ML, MR, NE, SN, TD, TG).

(63) Related by continuation (CON) or continuation-in-part
(CIP) to earlier application:
US 10/324,967 (CON)
Filed on 20 December 2002 (20.12.2002)

(71) Applicant (*for all designated States except US*): UNIVER-
SITY OF GENEVA [CH/CH]; 15, rue Lombard, CH-1205
Geneva (CH).

Published:

- with international search report
- before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): COSSON, Pierre
[FR/CH]; 55, chemin Poluzzi, CH-1227 Carouge (CH).
KÖHLER, Thilo [CH/CH]; 15, rue de la Tambourine,
CH-1227 Carouge (CH). BENGHEZAL, Mohammed
[CH/CH]; 8A, chemin des Fins, CH-1218 Grand-Saon-
nex (CH). MARCHETTI, Anna [CH/CH]; 36, avenue
Theodore-Weber, CH-1208 Geneva (CH). VAN DELDEN,

(88) Date of publication of the international search report:
2 September 2004

*For two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.*

(54) Title: PSEUDOMONAS AERUGINOSA AND KLEBSIELLA VIRULENCE GENES, PROTEINS, AND THEIR USE

(57) Abstract: A series of genes from *Pseudomonas aeruginosa* and *Klebsiella* are shown to encode products that are implicated in virulence. The identification of these genes therefore allows attenuated microorganisms to be produced. Furthermore, the genes or their encoded products can be used to identify antimicrobial drugs, diagnostic methods for the identification of a pathogen-associated disease, and in the manufacture of vaccines.

WO 2004/057018 A3

INTERNATIONAL SEARCH REPORT

Application No

PCT/CH 03/00836

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/21 C07K14/26 C12Q1/18 C12Q1/68 A61K39/104
A61K39/108

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12Q A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EP0-Internal, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	COSSON P ET AL: "PSEUDOMONAS AERUGINOSA VIRULENCE ANALYZED IN A DICTYOSTELIUM DISCOIDEUM HOST SYSTEM" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 184, no. 11, June 2002 (2002-06), pages 3027-3033, XP001146553 ISSN: 0021-9193 the whole document	1-6, 10-24, 26,27, 29-31, 33,34
X	WO 02/101081 A (PACCAUD JEAN-PIERRE ; COSSON PIERRE (CH); UNIV GENEVE (CH); KOHLER) 19 December 2002 (2002-12-19) cited in the application the whole document	1-6, 10-24, 26,27, 29-31, 33,34

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

25 March 2004

Date of mailing of the international search report

12. 07. 2004

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Hix, R

INTERNATIONAL SEARCH REPORT

I Application No

PCT/CH 03/00836

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	STOVER C K ET AL: "COMPLETE GENOME SEQUENCE OF PSEUDOMONAS AERUGINOSA PA01, AN OPPORTUNISTIC PATHOGEN" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 406, no. 6799, 31 August 2000 (2000-08-31), pages 959-964, XP000996980 ISSN: 0028-0836 the whole document	1-6, 10-24, 26,27, 29-31, 33,34
X	----- ESSAR D W ET AL: "DNA SEQUENCES AND CHARACTERIZATION OF FOUR EARLY GENES OF THE TRYPTOPHAN PATHWAY IN PSEUDOMONAS-AERUGINOSA" JOURNAL OF BACTERIOLOGY, vol. 172, no. 2, 1990, pages 853-866, XP008029001 ISSN: 0021-9193 the whole document	1-6, 10-24, 26,27, 29-31, 33,34
A	----- CHOI J Y ET AL: "Identification of virulence genes in a pathogenic strain of pseudomonas aeruginosa by representational difference analysis" JOURNAL OF BACTERIOLOGY, WASHINGTON, DC, US, vol. 184, no. 4, February 2002 (2002-02), pages 952-961, XP002972639 ISSN: 0021-9193 the whole document	
P,X	----- WO 03/022881 A (TUEMMLER BURKHARD ;RITZKA MARGIT (DE); KIEWITZ CLAUDIA (DE); LARBI) 20 March 2003 (2003-03-20) the whole document -----	1-27, 29-31, 33,34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CH 03/00836

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.: 25 and 28
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 25 and 28 involve a mammalian subject wherein said subject is a human, which is considered by this Authority to be covered by the provisions of Rule 67.1(ii) PCT. and Article 34(4)(a)(i) PCT.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
claims 1-6, 10-24, 26, 27, 29-31, 33-34 (partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Claims Nos.: 25 and 28

Claims 25 and 28 involve a mammalian subject wherein said subject is a human, which is considered by this Authority to be covered by the provisions of Rule 67.1(ii) PCT. and Article 34(4)(a)(i) PCT.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1:

Claims 1-6, 10-24, 26, 27, 29-31, 33-34 (partially):
An attenuated bacterial mutant *Pseudomonas aeruginosa* derived from a pathogenic bacterial strain wherein said attenuated mutant has a mutation of a gene VIR1 and reduced inhibition of *Dictyostelium amoeba* growth when compared to the growth observed in the presence of an isogenic bacterial strain. A method for identifying an antimicrobial drug comprising contacting a candidate composition with at least one polypeptide or polynucleotide encoded by the gene VIR1. Method for determining the degree of virulence of a pathogen in a subject comprising measuring the level of expression of at least one polypeptide or poly nucleotide encoded by the gene VIR1. A vaccine comprising a pharmaceutically effective dose of one or more of said attenuated bacterial mutants. A bacterial strain comprising an operon encoding a gene VIR1.

Inventions 2 to 19:

Claims 1-6, 10-24, 26, 27, 29-31, 33-34 (partially):
An attenuated bacterial mutant *Pseudomonas aeruginosa* derived from a pathogenic bacterial strain wherein said attenuated mutant has a mutation of a gene VIR2 to VIR19 and reduced inhibition of *Dictyostelium amoeba* growth when compared to the growth observed in the presence of an isogenic bacterial strain. A method for identifying an antimicrobial drug comprising contacting a candidate composition with at least one polypeptide or polynucleotide encoded by the gene VIR2 to VIR19. Method for determining the degree of virulence of a pathogen in a subject comprising measuring the level of expression of at least one polypeptide or poly nucleotide encoded by the gene VIR2 to VIR19. A vaccine comprising a pharmaceutically effective dose of one or more of said attenuated bacterial mutants. A bacterial strain comprising an operon encoding a gene VIR2 to VIR19.

Inventions 20 to 46: Claims 1-3, 7-24, 26, 27, 29-31, 33-34 (partially):

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

An attenuated bacterial mutant *Klebsiella* derived from a pathogenic bacterial strain wherein said attenuated mutant has a mutation of a gene VIR20 to VIR46 and reduced inhibition of *Dictyostelium amoeba* growth when compared to the growth observed in the presence of an isogenic bacterial strain. A method for identifying an antimicrobial drug comprising contacting a candidate composition with at least one polypeptide or polynucleotide encoded by the gene VIR20 to VIR46. Method for determining the degree of virulence of a pathogen in a subject comprising measuring the level of expression of at least one polypeptide or poly nucleotide encoded by the gene VIR20 to VIR46. A vaccine comprising a pharmaceutically effective dose of one or more of said attenuated bacterial mutants. A bacterial strain comprising an operon encoding a gene VIR20 to VIR46.

Invention 47: Claim 32:

An attenuated bacterial mutant derived from a pathogenic bacterial strain wherein said attenuated mutant has a mutation of a gene selected from the group consisting of pchE, pchF, pchG, pchH and pchI and reduced inhibition of *Dictyostelium amoeba* growth when compared to the growth observed in the presence of an isogenic bacterial strain.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/CH 03/00836

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 02101081	A	19-12-2002	CA 2450671 A1 EP 1395671 A2 WO 02101081 A2	19-12-2002 10-03-2004 19-12-2002
WO 03022881	A	20-03-2003	WO 03022881 A2 EP 1425300 A2	20-03-2003 09-06-2004